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Responsiveness of human lung cancer/nude mouse to antitumor agents in a model using clinically equivalent doses*

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Summary. The responses of 14 lines of human lung cancer xenografts in BALB/c-nu/nu mice to eight known antitumor agents were investigated. These xenografts consisted of four small-cell carcinomas (SCLC) and ten non-small-cell carcinomas (four large cell, three squamous cell, and three adenocarcinomas; NSCLC). The doses used in the experiments were the maximum tolerated dose (MTD) in nude mice and the "rational dose" (RD), the latter considered to be pharmacokinetically equivalent to the clinical dose. When given at MTDs, all drugs except 5-fluorouracil (5-FU) and methotrexate (MTX) were extremely effective against NSCLC as well as SCLC. The response rates of drug-sensitive SCLC to mitomycin C (MMC), ACNU, and vinblastine (VLB) were 100%, and those to Adriamycin (ADR) and vincristine (VCR) were 75%. In addition, the response rates of even drug-resistant NSCLC to MMC and VLB were 70% and 90%, respectively. In contrast, the response rates of NSCLC to RDs of the drugs were reduced to <40% and corresponded well to the respective clinical rates. In SCLC, a good correlation of experimental and clinical response rates was observed with four drugs [cyclophosphamide (CPM), ACNU, VLB, and 5-FU]. As a result, we emphasize that a more reasonable prediction of the clinical effectiveness of antitumor agents can be made by a protocol using clinically equivalent doses.

Intr duction

Nude mice are very useful for preclinical studies in cancer chemotherapy because of their high take-rates of human tumor specimens. A number of studies concerning the prediction of clinical response to antitumor agents using the human tumor/nude mouse system have been reported [2, 3, 7, 10, 14, 15]; in most of them the protocols for experiments, such as dose, route of treatment, and evaluation, were almost the same as those used for murine tumors. For example, optimal doses in mice were given i.p., and the effect was evaluated in terms of the lowest T/C (%) value of tumor size throughout the observation period. However, considering the pharmacokinetic differences of agents in humans vs mice, more reasonable protocols should be designed to predict the clinical effect of any candidate compounds, based on results obtained by the human tumor/nude mouse system. Since each tumor originating from the same kind of organ has individual sensitivity to an antitumor agent, it would not be reasonable to predict clinical effectiveness of the agent from its effect on a single tumor line. Rather, a response rate based on several lines should be used as the index of effectiveness on one type of tumor.

In our previous paper [5], we compared plasma levels of currently used antitumor agents in humans vs nude mice and determined the doses in nude mice that would reproduce plasma levels of the drugs in patients given therapeutically effective doses; we termed this dose the "rational dose" (RD). In a subsequent paper [6], good correlation was demonstrated between the therapeutic results of 11 human gastric tumor xenografts treated with 8 typical antitumor agents at their respective RDs and the reported clinical response rates to those drugs.

The aim of the present studies was to determine whether such correlation with clinical results holds true not only for gastric cancer but for lung cancer as well. A number of human lung cancer xenografts were similarly treated with typical antitumor agents at their MTDs and RDs, and their response rates were compared with clinical rates. If the correlation between the human and nude mouse are confirmed for lung cancer as well as other types of malignancy, such an approach would be useful in finding tumor-type-specific agents.

Lung cancer is classified into several histological types, each of which exhibits differential sensitivities to antitumor agents. The general classification basically involves four types: small-cell, large-cell, and squamous-cell carcinoma and adenocarcinoma. The latter three types are taken together as non-small-cell lung cancer (NSCLC) in contrast to small-cell lung cancer (SCLC) from the standpoint of drug sensitivity; therefore, in this study comparison was carried out independently, based on this rough classification.

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Abbreviations: SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer; MTD, maximum tolerated dose; RD, rational dose; MMC, mitomycin C; CPM, cyclophosphamide; ACNU, 1-(4-amino-2-methylpyrimidin-5-yl)-methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride; ADR, Adriamycin; VCR, vincristine; VLB, vinblastine; 5-FU, 5-fluorouracil; MTX, methotrexate

Materials and methods

Antitumor agents. MMC, ADR, and 5-FU were kindly supplied by Kyowa Hakko Kogyo Co. (Tokyo), CPM, by Shionogi & Co. (Osaka), and ACNU, by Sankyo Co., Ltd. (Tokyo, Japan), as pure crystals for experimental use. Both VCR and VLB were purchased from Shionogi & Co., and MTX was obtaind from Lederle Japan, Ltd. (Tokyo) in a form for clinical use.

All drugs were dissolved in 0.85% NaCl solution immediately before treatment. The MTDs of the drugs were determined as maximal nonlethal doses by a single i.v. injection (for 5 consecutive days only in the case of 5-FU and MTX) of several doses, increased at a constant ratio of 1.2.

The RD of each drug was determined to be pharmacokinetically equivalent to the clinical dose in the previous study [5]. Briefly, some graded doses of each drug were injected i.v. into nude mice and their plasma were collected at several time points. The plasma levels of each drug were assayed according to the methods as follows: MMC and 5-FU, by microbiological assay; ADR, ACNU, and VLB, by high-pressure liquid chromatography (HPLC); VCR, as total radioactivity of [^{3}H]VCR; MTX, by dihydrofolate reductase inhibition assay; and CPM, by fluorometry of metabolically produced acrolein. Plasma clearance curves thus obtained were compared with those of human patients given a clinical dose of the respective drug, taken from the literature. The dose most closely reproducing clinical plasma levels in nude mice was determined to be the clinically equivalent dose (RD).

Human tumor xenograft lines. A total of 14 human lung cancers established as xenografts in nude mice were used. Histologically, four lines belonged to the small-cell type, and the other ten lines were non-small-cell type. More detailed histological types as well as growth rates and prior chemotherapy are given in Table 1. LX-1 was kindly supplied by the National Cancer Institute, Bethesda, Md; and Lu-24, Lu-130, Lu-134, Lu-99, Lu-116, Lu-65, and Lu-61 were furnished by Dr. Y. Shimosato, National Cancer Center Research Institute, Tokyo, Japan. QG-56 was established by the inoculation of cells from a cultured line, QG-56 [16]. The other lines were established by the Central Institute for Experimental Animals. These tumors were maintained by serial s.c. transplantation of a (2-mm^3) fragment into the right subaxillary region of female athymic BALB/c-nu/nu mice (CLEA Japan, Inc., Tokyo) every 3–8 weeks according to their respective growth rates.

The animals (6–8 weeks old, 20–25 g body weight) were housed in ethylene oxide-gas-sterilized and filter-capped polycarbonate cages with autoclaved bedding and were fed ^{60}Co-irradiated (3 Mrad) food and autoclaved water ad libitum. All cages were kept in laminar air-flow units.

Chemotherapy. The growth of tumors was observed; after they became palpable, the size of each tumor was measured using calipers. Tumor volume (V) was calculated according to the formula

$$V = 1/2 \times a \times b^2,$$

a and b being the tumor length and width (in mm), respectively. When the tumor volume reached 100–300 mm^3, mice bearing such tumors were randomized into groups consisting of six mice each and drug treatment was started. MTDs and RDs determined in advance were given i.v. as a single injection, except those of 5-FU and MTX, which were given daily for 5 consecutive days; these schedules generally simulate one course of practical therapy in the clinic. Tumor sizes were measured twice a week, and the tumor volume was then expressed as relative tumor volume (RV):

$$RV = V_n/V_0,$$

where V_n is the tumor volume at day n and V_0 is the initial volume immediately before the treatment was started (day 0). The mice were observed for at least 3–4 weeks; on the disappearance of a tumor the observation was continued for about 2 months.

Evaluation. On any experimental day, T/C (%) was expressed as the average RV of the treated group vs that of controls. The effectiveness of each drug was evaluated by the statistical significance of RV values between both groups as determined by the Mann-Whitney U-test ($P < 0.01$; one-sided) as well as the T/C (%) values on day 14, the effective criterion for which was $\leq 50\%$.

Results

Characteristics of each xenograft line are listed in Table 1. The upper four lines are SCLC, and the lower ten lines are NSCLC, which comprise four large-cell and three squamous-cell carcinomas and three adenocarcinomas. Among the SCLC lines, only LX-1 grew rapidly after a latent period of about 10 days; the latency period of the other three lines was about 1 month. Patients from whom Lu-130, and Lu-134 cells had been obtained had received prior therapy, albeit not extensive. Large-cell carcinomas began to grow earlier; their latent periods were < 2 weeks, and their tumor sizes reached 20- to 30-fold the initial volume. The patient bearing the Lu-116 line was treated once with MMC (10 mg) prior to surgery. Most of the squamous-cell carcinomas and adenocarcinomas began to grow 2–3 weeks after inoculation and grew rather slowly.

Each tumor was treated with VLB at its MTD (11 mg/kg) and RD (2.6 mg/kg); the changes in relative growth rates (T/C%) vs the days after treatment are plotted in

Table 1. Profiles of human lung tumor xenograft lines

	Histology	Tumor line	Prior chemo-therapy	Tumor mass--doubling time (days)
SCLC	Small-cell	LX-1	–	4.0
		Lu-24	–	5.0
		Lu-130	+	6.7
		Lu-134	+	7.0
NSCLC	Large-cell	Lu-116	+	3.6
		Lu-99	–	4.0
		Lu-65	–	4.8
		LC-6	–	6.3
	Squamous-cell	Lu-61	–	7.3
		QG-56		6.6
		LC-1	–	4.8
	Adenocarcinoma	L-27		4.6
		LC-11	–	7.3
		LC-17	–	7.3

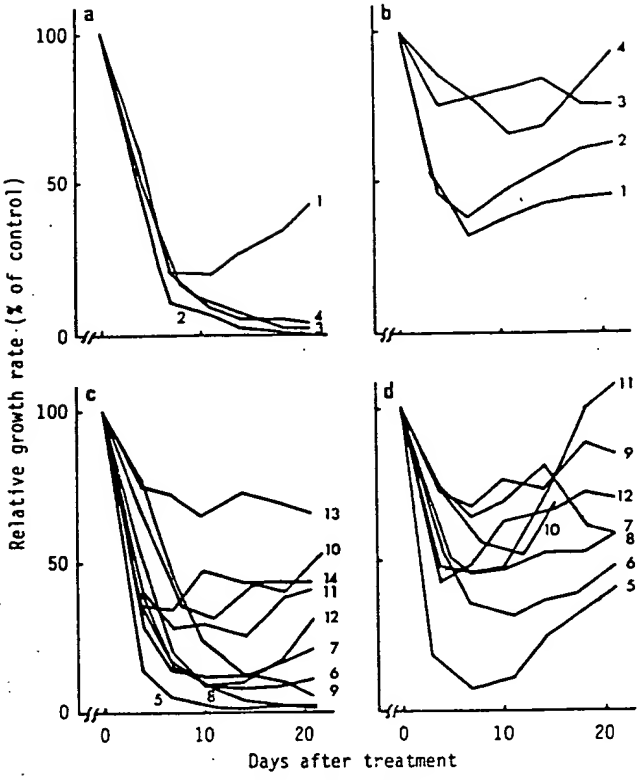

Fig. 1. Response of human lung cancer xenograft lines to the MTD and RD of VLB. VLB was injected i.v. at the MTD (*a*, *c*) and RD (*b*, *d*) when the tumor volume reached 100–300 mm^3. The relative tumor growth rate (T/C %) of each line is plotted against the days after treatment. *a*, *b*, SCLC; *c*, *d*, NSCLC: *1*, LX-1; *2*, Lu-24; *3*, Lu-130; *4*, Lu-134; *5*, Lu-116; *6*, Lu-99; *7*, Lu-65; *8*, LC-6; *9*, Lu-61; *10*, QG-56; *11*, LC-1; *12*, L-27; *13*, LC-11; *14*, LC-17

Fig. 1. All SCLC lines markedly responded to the treatment with the MTD, and the Lu-24 xenograft completely disappeared (Fig. 1, *a*). Nine of ten NSCLC lines responded to this drug, and the T/C value of four lines was reduced to <10% (Fig. 1, *c*). On the other hand, when treated with the RD (ca. ¼ of the MTD) of VLB, only a few lines responded in both SCLC and NSCLC xenografts (Fig. 1 *b*, *d*).

All lung cancer lines were similarly treated with other antitumor agents at their respective MTDs and RDs; the results are listed in Tables 2–5. In Table 2, responses of NSCLC lines to the MTDs of eight antitumor agents are shown. Generally speaking, NSCLCs are not clinically responsive to antitumor agents, but all of the drugs except 5-FU and MTX were very effective against large-cell carcinomas. At day 14, a T/C value of <10% was very often observed, and several mice were finally cured. All squamous-cell carcinomas except QG-56 also responded to several drugs. In contrast, adenocarcinomas were mostly unresponsive, even to MTDs of these agents.

Therapeutic results with the RD of each drug against NSCLCs are shown in Table 3. In comparison with those responsive to MTDs, the number of lines responsive to the RDs of these agents was markedly reduced. Among the ten lines, only three responded to more than two drugs. Only ACNU showed T/C values of <10% against Lu-116 and LC-6, and in other effective cases, these values were 30%–50%. Against QG-56 and three adenocarcinomas, L-27, LC-11, and LC-17, no agent was effective at its RD.

Data on the SCLCs are demonstrated in Tables 4 and 5. Among the four lines tested, Lu-24, Lu-130, and Lu-134 were very sensitive to antitumor agents at the MTDs. T/C values of <10% were often observed, and in several cases complete regression was recognized. LX-1 was less sensitive than the others, responding only to MMC, ACNU, and VLB. None of the lines responded to 5-FU and MTX, and CPM was effective only against Lu-24. Although SCLC is clinically classified as a drug-sensitive cancer, at the RDs none of the drugs were very effective against these xenografts, as shown in Table 5; no T/C value of <10% was obtained. The most sensitive line was Lu-24, and it responded to MMC, CPM, ADR, and VLB; in contrast, LX-1 responded only to MMC.

Overall response rates of NSCLCs and SCLCs to the MTD and RD of each drug are shown in Tables 6 and 7, respectively, compared with clinical response rates. As shown in Table 6, the response rates of all NSCLCs except 5-FU and MTX to the MTDs of the drugs were >40%, clearly much higher than the clinical rates. In contrast, those to the RDs were reduced and mostly in accordance with the clinical rates. Similarly, the response rates of SCLCs to the MTDs of the drugs were unreasonably high-

Table 2. Response of human NSCLC xenografts implanted in nude mice to MTDs of antitumor agents

Drug	Dose (mg/kg)	Relative growth rate (T/C %)[a]									
		Lu-116	Lu-99	Lu-65	LC-6	Lu-61	QG-56	LC-1	L-27	LC-11	LC-17
MMC	6.7	5[b]	12	5	30 (3)[c]	14	60	21	52	49	6
CPM	260	8	10	4	21	61	64	31	57	71	51
ACNU	48	0 (6)	25	2	0 (6)	13	58	54	57	76	81
ADR	12	35	72	47	31	78	55	50	59	63	68
VCR	1.6	2	18	27	40	86	99	58	72	85	122
VLB	11	1 (2)	8	13	4 (2)	13	43	25	10	73	43
5-FU	19 × 5	62	46	70	100	105	72	66	91	79	67
MTX	15 × 5	85	85	83	38	100	104	97	105	92	103

[a] Determined as the ratio (%) of mean relative tumor volume in the treated group to that in the nontreated group at day 14 (see *Materials and methods*)

[b] Underlined values are "effective" according to our evaluation criterion: T/C value of ≤50%, statistically significant according to the Mann-Whitney U-test ($P < 0.01$; one-sided)

[c] Numbers in parenthesis represent the number of mice cured during the experimental period

Table 3. Response of human NSCLC xenografts implanted in nude mice to RDs of antitumor agents

Drug	Dose (mg/kg)	Relative growth rate (T/C %)[a]									
		Lu-116	Lu-99	Lu-65	LC-6	Lu-61	QG-56	LC-1	L-27	LC-11	LC-17
MMC	1.7	109	64	65	87	61	72	70	(–)[c]	92	62
CPM	65	78	41	54	66	(–)	(–)	124	(–)	(–)	(–)
ACNU	8+2+0.8[d]	9[b]	86	85	0 (6)[e]	50	97	88	(–)	(–)	(–)
ADR	12	35	72	47	31	78	55	50	59	63	68
VCR	0.4	72	58	76	79	(–)	(–)	86	(–)	(–)	(–)
VLB	2.6	24	36	81	52	73	69	71	65	(–)	108

[a] Determined as the ratio (%) of mean relative tumor volume in the treated group to that in the nontreated group at day 14 (see *Materials and methods*)
[b] Underlined values are "effective" according to our evaluation criterion: T/C value of ≤50%, statistically significant according to the Mann-Whitney U-test ($P < 0.01$; one-sided)
[c] Judged to be "ineffective" from data on responses to MTD (Table 2)
[d] Given as 3 intermittent injections of 8 (0 min), 2 (25 min), and 0.8 mg/kg (70 min) on 1 day
[e] Numbers in parenthesis represent the number of mice cured during the experimental period

Table 4. Response of human SCLC xenografts implanted in nude mice to MTDs of antitumor agents

Drug	Dose (mg/kg)	Relative growth rate (T/C %)[a]			
		LX-1	Lu-24	Lu-130	Lu-134
MMC	6.7	14[b]	1 (2)[c]	5	6
CPM	260	66	1 (2)	87	90
ACNU	48	5	5	2	2 (1)
ADR	12	69	12	41	40
VCR	1.6	72	2 (3)	16	14
VLB	11	27	2 (6)	7	5
5-FU	19×5	60	95	78	93
MTX	15×5	66	109	75	80

[a] Determined as the ratio (%) of mean relative tumor volume in the treated group to that in the nontreated group at day 14 (see *Materials and methods*)
[b] Underlined values are "effective" according to our evaluation criterion: T/C value of ≤50%, statistically significant according to the Mann-Whitney U-test ($P < 0.01$; one-sided)
[c] Numbers in parenthesis represent the number of mice cured during the experimental period

Table 5. Response of human SCLC xenografts implanted in nude mice to RDs of antitumor agents

Drug	Dose (mg/kg)	Relative growth rate (T/C %)[a]			
		LX-1	Lu-24	Lu-130	Lu-134
MMC	1.7	39[b]	30	57	49
CPM	65	(–)[c]	41	(–)	(–)
ACNU	8+2+0.8[d]	54	115	14	19
ADR	12	69	12	41	40
VCR	0.4	97	115	94	86
VLB	2.6	53	42	84	68

[a] Determined as the ratio (%) of mean relative tumor volume in the treated group to that in the nontreated group at day 14 (see *Materials and methods*)
[b] Underlined values are "effective" according to our evaluation criterion: T/C value of ≤50%, statistically significant according to the Mann-Whitney U-test ($P < 0.01$; one-sided)
[c] Judged to be "ineffective" from data on responses to MTD (Table 4)
[d] Given as 3 intermittent injections of 8 (0 min), 2 (25 min), and 0.8 mg/kg (70 min) on 1 day

Table 6. Comparison of experimental and clinical response rates of human NSCLC xenografts to various antitumor agents

Antitumor agent	Experimental response rate (%)		Clinical response rate (%)	Reference
	MTD	RD		
MMC	70 (7/10)	0 (0/10)	20 (23/115)	[1]
CPM	50 (5/10)	10 (1/10)	8 (30/369)	[1]
ACNU	50 (5/10)	30 (3/10)	13 (8/64)	[11]
ADR	40 (4/10)	40 (4/10)	13 (34/261)	[1]
VCR	40 (4/10)	0 (0/10)	10 (8/84)	[1]
VLB	90 (9/10)	20 (2/10)	27 (6/22)	[1]
5-FU	10 (1/10)	≥10 (1/10)	9 (3/34)	[1]
MTX	10 (1/10)	≥10 (1/10)	10 (25/247)	[1]

er than the clinical rates, except those of CPM, 5-FU, and MTX, as shown in Table 7: three drugs (MMC, ACNU, and VLB) showed 100% response rates (4/4), and the rest (ADR and VCR), 75% (3/4). The response rates of SCLCs to the RDs are also given in this table. For CPM, ACNU, VLB, and 5-FU, the response rates at the RDs were similar to the clinical rates. On the other hand, those for MMC and ADR were higher, whereas those for VCR and MTX were much lower than the clinical rates.

Discussion

In our new protocol, we emphasized the following two points: the use of doses pharmacokinetically equivalent to the clinical dose, and giving priority to statistical significance in evaluating the effectiveness. However, when the evaluation was made based on only the significance, even T/C values of 70%–80% would be occasionally judged to be "effective". Therefore, we set the upper effective limit of T/C at 50%, because most data <50% were significant.

We measured tumor sizes twice a week and observed tumor-bearing mice for longer than 3 weeks. Growth rates of the tumor lines were not uniform. Regardless of the difference in growth rates, the significance at day 14 was most universal during the experimental period; thus, we selected day 14 as the day of evaluation.

Treatment of lung cancer is quite complicated, because this disease shows variety in histology, growth rate, dis-

Table 7. Response rate of human SCLC xenografts to various antitumor agents

Antitumor agent	Experimental response rate (%)		Clinical response rate (%)	Reference
	MTD	RD		
MMC	100 (4/4)	75 (3/4)	50 (17/34)	[9]
CPM	25 (1/4)	25 (1/4)	32 (98/307)	[12]
ACNU	100 (4/4)	50 (2/4)	47 (14/30)	[11]
ADR	75 (3/4)	75 (3/4)	27 (23/86)	[12]
VCR	75 (3/4)	0 (0/4)	42 (18/43)	[4]
VLB	100 (4/4)	25 (1/4)	33 (3/9)	[13]
5-FU	0 (0/4)	≧0 (0/4)	12 (3/24)	[8]
MTX	0 (0/4)	≧0 (0/4)	26 (29/110)	[12]

semination, and other factors [12]. Among these cancers, SCLC is well known to be the most rapidly growing and disseminating histological type, and many cases are inoperable at diagnosis. However, with regard to drug sensitivity, SCLC is classified as a comparatively sensitive type, and systemic chemotherapy is extensively given for the treatment of SCLC. On the other hand, NSCLCs, including large-cell and squamous-cell carcinomas and adenocarcinoma, are confined for considerably longer to the primary region and are thus resectable by surgery in the early stage. In the advanced stage, however, the management of NSCLC should rely on chemotherapy or radiotherapy. Unfortunately, at this stage many cases do not respond to most of the drugs currently used, at least as single agents, and NSCLC is generally categorized as a drug-resistant cancer.

The object of the present study was to confirm the usefulness of this new protocol, using the human tumor/nude mouse system to evaluate the antitumor effect of candidate compounds. As demonstrated by the example in Fig. 1 and the data in Tables 2 and 4, when treated with the MTDs for nude mice, the doses often used in common screening protocols, not only drug-sensitive SCLC but also drug-resistant NSCLC responded remarkably to most of the drugs tested. Only adenocarcinomas and one squamous-cell carcinoma (QG-56) were not so responsive.

In the case of clinical therapy, human MTDs of the drugs were given repeatedly every 3–4 weeks. Taking this into consideration, the overall response rates of NSCLC as well as SCLC to the nude mouse MTDs of most these drugs were much higher than the clinical rates, indicating that testing at the MTD might result in an overestimation of drug efficacy.

We also tried to select the dose of each drug that would reproduce in nude mice the plasma level of the clinical dose in humans (its RD). In general, RDs were 4- to 5-fold lower than MTDs, whereas that of ADR was the same as its MTD. Those of 5-FU and MTX were higher than their MTDs, which means that nude mice cannot be treated at real RDs of these agents. When treated with RDs, the responses of NSCLCs markedly decreased (Fig. 1 and Table 3). T/C (%) values increased, and an overall response rate to each agent was generally comparable with the respective clinical rate, suggesting the reasonableness of using RDs instead of MTDs in NSCLC.

Effect of the drugs on SCLCs were necessarily reduced when the tumors were treated with RDs. Only ACNU (against 2 lines) and ADR (against Lu-24) retained a T/C value of $<20\%$ (Table 5). As a result, in SCLCs a satisfactory coincidence of the experimental response rates to the RDs with the clinical rates were observed in four of eight agents (Table 7). Although VCR is one of the drugs most often used in the treatment of SCLC, no line responded to this drug at the RD, which was determined in terms of the plasma level of the total radioactivity of [^{3}H]-VCR [5]. As VCR is known to bind to plasma protein, we should determine its RD based on the plasma level of free drug. The plasma levels of 5-FU and MTX in nude mice treated even with the respective MTDs were lower than those in humans treated with clinically therapeutic doses [5]. Thus, the experimental response rates to these drugs are expected to be rather lower than the clinical response rates.

Another probable reason for the low correlation between the experimental and clinical response rates in SCLC would be the low number of available tumor lines. As the response of each line to a drug has much greater influence on the overall response rate when the number of lines is small, we should use as many tumor lines as possible to confirm the correlation of response rates between human tumor xenografts and patients. Considering the present experiment, use of approximately ten lines of each type of tumor would be desirable for proper prediction, albeit without a statistical basis. Nevertheless, it is obvious that more reasonable results were observed even in SCLC when the tumors were treated with RD than with MTD. Parallel to our studies on lung cancer, we also carried out similar experiments with human gastric and breast tumor xenograft lines, and good correlations between experimental and clinical responses were observed [6].

When a new drug is tested in the preclinical phase, it cannot be tested at the RD, as no human data would yet be available. Therefore, it can only be evaluated against various tumor types at the MTD in nude mice as well as doses such as ½ and ¼ of the MTD. However, once the drug enters clinical phase I study, plasma levels in humans and nude mice can be compared to determine which dose corresponds to the RD, and the response rates of each tumor type to the RD can be determined based on the therapeutic data obtained in advance. We can then predict that the drug might be clinically effective against tumor types with higher response rates.

Recent progress in pharmacokinetics is remarkable. When pharmacokinetic parameters such as the total body clearance and distribution volume of any drug in several animal species are available, the prediction of those values in humans is made possible by the animal scale-up methods. Using a similar method, the human MTD can also be predicted using animal MTDs. Thus, it is expected that we can estimate the RD of a candidate drug and predict its clinical effect in the preclinical stage.

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References

1. Bakowski MT, Crouch JC (1983) Chemotherapy of non-small cell lung cancer: a reappraisal and a look to the future. Cancer Treat Rev 10: 159

2. Giovanella BC, Stehlin JS, Shepard RC, Williams LJ (1983) Correlation between response to chemotherapy of human tumors in patients and in nude mice. Cancer 52: 1146
3. Giuliani FC, Zirvi KA, Kaplan NO (1981) Therapeutic response of human tumor xenografts in athymic mice to doxorubicin. Cancer Res 41: 325
4. Hansen HH (1980) Management of small cell anaplastic carcinoma. In: Lung cancer. Excerpta Medica, Amsterdam, p 113
5. Inaba M, Kobayashi T, Tashiro T, Sakurai Y (1988) Pharmacokinetic approach to rational therapeutic doses for human tumor-bearing nude mice. Jpn J Cancer Res 79: 509
6. Inaba M, Tashiro T, Kobayashi T, Sakurai Y, Maruo K, Ohnishi Y, Ueyama Y, Nomura T (1988) Responsiveness of human gastric tumors implanted in nude mice to clinically equivalent doses of various antitumor agents. Jpn J Cancer Res 79: 517
7. Inoue K, Fujimoto S, Ogawa M (1983) Antitumor efficacy of seventeen anticancer drugs in human breast cancer xenograft (MX-1) transplanted in nude mice. Cancer Chemother Pharmacol 10: 182
8. Joss RA, Cavalli F, Goldhirsch A, Brunner K (1986) New drugs in small-cell lung cancer. Cancer Treat Rev 13: 157
9. Ota K, Nishimura M (1980) Present status of lung cancer chemotherapy in Japan. In: New anticancer drugs. Springer-Verlag, Berlin Heidelberg, p 167 (Recent Results in Cancer Research, vol 70)
10. Ovejera AA, Houchens DP, Barker AD (1977) Sensitivity of a human tumor xenograft in nude (athymic) mice to various clinically-active drugs. In: Proceedings of the 2nd International Workshop on Nude Mice. University of Tokyo Press, Tokyo, p 451
11. Saijo N, Niitani H (1980) Experimental and clinical effect of ACNU in Japan, with emphasis on small-cell carcinoma of the lung. Cancer Chemother Pharmacol 4: 165
12. Sarna GP, Holmes EC, Petrovich Z, Figlin RA (1985) Lung cancer. In: Cancer treatment, 2nd edn. WB Saunders, Philadelphia, p 180
13. Selawry OS (1973) Monochemotherapy of bronchogenic carcinoma with special reference to cell type. Cancer Chemother Rep 4 (3): 177
14. Steel GG, Courtenay VD, Peckham MJ (1983) The response to chemotherapy of a variety of human tumor xenografts. Br J Cancer 47: 1
15. Taetle R, Rosen F, Abramson I, Venditti J, Howell S (1987) Use of nude mouse xenografts as preclinical drug screens: in vivo activity of established chemotherapeutic agents against melanoma and ovarian carcinoma xenografts. Cancer Treat Rep 71: 297
16. Yasumoto K, Ohta M, Nomoto K (1976) Cytotoxic activity of lymphocytes to bronchogenic carcinoma cells in patients with lung cancer. Gann 67: 505

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